United States Department of the Interior

WESTERN FISHERIES RESEARCH CENTER
BIOLOGICAL RESOURCES DIVISION
U.S. GEOLOGICAL SURVEY
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28 June 2006; revised 25 July 2006

A. Title: Application for Permit for Scientific Purposes under the Endangered Species Act of 1973.
Project Name: Importance of lake or estuarine habitats for juvenile rearing in Puget Sound populations of Chinook salmon.

B. Species: Puget Sound Chinook salmon (Lake Washington drainage and nine hatchery populations)

C. Date of Permit Application: 29 June 2006; revised 25 July 2006

D. Applicant Identity:
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E. Information on Personnel, Cooperators, and Sponsors:
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Cooperators/collaborators:

Sayre Hodgson, Nisqually Tribe, 360-438-8687
Marian Bailey, US Fish and Wildlife Service, 360-259-8022
Chris Ellings, Ducks Unlimited & Nisqually National Wildlife Refuge, 360-753-9467
Nick Gayeski and Brian Kennedy, Washington Trout, 425-788-1167 x225
Bruce Bachen, Seattle Public Utilities, 206-684-7935
Roger Tabor, US Fish and Wildlife Service, 360-753-9541
Greg Volkhardt, Washington Dept. Fish and Wildlife, 360-902-2779
Fred Goetz, U.S. Army Corps of Engineers, 206-764-3515

Disposition of samples:

Otoliths and scales will be retained indefinitely at Western Fisheries Research Center. Samples from fish collected in the Cedar River/Lake Washington system will be shared with Nick Gayeski and Brian Kennedy (see above for contact information). We will canvas associates (e.g., personnel at NOAA Fisheries, Northwest Science Center; staff at University of Washington) and cooperators for any interest in the remains other than scales and otoliths. If no one desires the remains, the remains will be autoclaved and disposed according to standard laboratory procedures.

F. Project Description, Purpose, and Significance:

1. The purpose of our work is to improve the understanding of key rearing habitats for juvenile Chinook salmon in Puget Sound. The purpose of our work with Nisqually fish is to develop and use otolith microstructure analysis as a tool for characterizing the importance of the estuary to Chinook salmon in the Nisqually River before (now) and after restoration (future) at Nisqually National Wildlife Refuge. This tool would be used to quantify use of this habitat, including length of residence and growth, thereby providing a partial assessment of restoration benefits to federally threatened Chinook salmon. These results would inform and promote the effectiveness of future restoration efforts.

Similarly, the purpose of our work with Cedar River Chinook salmon is to determine the importance of rearing in Lake Washington compared to exclusive stream rearing in the Cedar River for the overall population so that managers have an improved basis for selecting the most cost-effective restoration actions. Our work complements WDFW's downstream migrant salmon production studies which include estimation of "lake/marine survival of natural production" by marking juvenile fish and subsequently recovering or detecting them. Our work complements these direct estimates of survival by showing the proportion of the adult salmon population (i.e., the survivors) that used the lake as juveniles. Both the Cedar River anadromous fish committee and the flow committee have identified this question as a key uncertainty

for planning protection and restoration efforts, and have requested and funded our work to complement the WDFW effort.
Our approach (otoliths) requires sacrifice or incidental mortality of juveniles rearing in the various habitats to provide baseline samples that illustrate the otolith microstructure from each habitat type. These baseline samples then are used to interpret the otolith microstructure of the adult salmon and assign a life history type to each adult (lake-reared vs exclusively stream-reared juvenile). We anticipate that lake-reared fish will exhibit increased growth (or alternative microchemistry) over exclusively stream-reared fish each year so that archived otoliths from adults of various brood years can be assessed without corresponding collections of juveniles from each brood.
See attached proposals/study plans.

2. This study does not respond to an official recommendation or requirement of a Federal agency.

3. This study has much broader significance than for the specific projects. The population response of Chinook salmon to estuary restoration is unknown. Evaluation of changes in estuary utilization after the massive estuary restoration occurring in the Nisqually River will help managers assess the utility of restoration for many other estuaries in Puget Sound and along the West Coast. The increased understanding of otolith analysis as a tool to evaluate the relative importance of various types of habitats (lake- or estuary- vs. river-rearing) to Chinook salmon populations should prove valuable to a broad range of restoration projects and listed populations.

4. We are collaborating with parties listed above. The Nisqually Tribe is doing the field collections for the Nisqually work under their Section 10 permit. We request approval to collect only hatchery fish from the various hatcheries whose fish occur in the study area (based on CWT recoveries) after they are released. Collections during 2006 were made under the Section 10 permits of several of these collaborators (Goetz; Volkhardt; Tabor); however, insufficient quotas were available to us to provide all the samples needed this year, and the available excess quota from their permits for 2007 are unknown. To the greatest extent possible we will use incidental mortalities from WDFW trapping operations to provide the needed samples in 2007. In "typical" years, incidental mortalities should provide the samples needed from Bear Creek and the Cedar River (see attached contract), requiring us to sacrifice only 40 juvenile fish from Lake Washington. Most or all of the fish that we obtain from the Lake Washington populations will be shared with Oregon Trout (Gayeski and Kennedy) who will analyze and evaluate the otolith microchemistry, similar to our approach with otolith microstructure, as an alternative method for discriminating between lake- and stream-rearing.

5. The listed species (Puget Sound Chinook salmon) must be used because our study is to provide information on the life history of Chinook salmon in the Nisqually River and Lake Washington systems, and no appropriate alternative species exist.

G. Project Methodology (see appended study plans for details):
Duration: February 2006 to March 2008. Juvenile salmon will be collected during February-July of 2007.
Capture methods and locations: Capture methods will consist of night snorkeling with small dip nets, beach seining, and downstream migrant trapping (trapping conducted by WDFW). The fish will be measured for length and weight, euthanized in buffered MS-222, stored in 95% ethanol, and their otoliths subsequently removed and processed at our laboratory according to standard practices (Stevenson and Campana 1992). Fish will be collected from Bear Creek (~ N 47° 45.5'; W 122° 09.5'), north Lake Washington beaches (~ N 47° 45.5'; W 122° 14.4'), south Lake Washington beaches (~ N 47° 30'; W 122° 13'), and various hatcheries (see list below). Fish will be collected from hatchery ponds by dip net and subsequently will be treated as just stated above.
The preferred source of fish from Bear Creek and Cedar River is incidental mortality at the WDFW traps deployed in those streams. "Typical years" should provide enough incidental mortalities to satisfy our request for stream-reared fish without any purposeful killing; however like 2006, some years provide very few such mortalities. Accordingly, my request includes stream-reared fish in case incidental mortalities are too few. Almost no incidental mortalities are expected from lake sampling by other investigators so I expect to kill the full 40 lake-reared fish that are requested.
No additional take of Puget Sound Chinook salmon will occur because any fish inadvertently killed during capture and handling will be part of the total take that I am requesting.

H. Description and Estimates of Take: See tables at the end of this application for numbers requested. The numbers of fish requested, 165 juvenile hatchery fish and 70 juvenile wild fish, are the minimum numbers that we judge suitable to provide reliable baselines for interpreting the otoliths from adult fish (carcasses) in archived collections or to be sampled by our cooperators. The recent trend (1996 – 2005) of Cedar River Chinook salmon is stable or increasing (page 3, Burton et al. 2005; Paul Faulds, Seattle Public Utilities (SPU), personal communication; Rand Little, SPU, personal communication including data shown in appended figure – {Paul Faulds reports 339 redds for 2005. This number of redds should translate to an escapement considerably larger than 339). Recent escapements to Bear Creek also are stable or increasing (see attached plot from data supplied by Rand Little, SPU). The numbers requested as take in this application are an extremely small proportion of the juvenile populations of hatchery and wild fish to be sampled.
We will sacrifice correspondingly fewer fish than requested when incidental mortalities appropriate for our use are available from WDFW's trapping on Bear Creek and Cedar River. Incidental mortalities are appropriate for our study if they are temporally distributed through the growing season with the majority taken in May and June. In a typical year, incidental mortalities should meet our entire requirement for stream-reared fish; however, this was not the case in 2006 and we wish to be prepared if similar conditions occur in 2007.
No USFWS listed species (e.g., bull trout) will be affected by our work.

I. Transportation and Holding

N/A

J. Cooperative Breeding Program

I would be willing to participate in a cooperative breeding program and to maintain or contribute data to a breeding program.

K. Previous or Concurrent Activities Involving Listed Species:

None

L. Certification:

"I hereby certify that the foregoing information is complete, true and correct to the best of my knowledge and belief. I understand this information is submitted for the purpose of obtaining a permit under the Endangered Species Act of 1973 (ESA) and regulations promulgated thereunder, and that any false statement may subject me to the criminal penalties of 18 U.S.C. 1001, or to penalties under the ESA."

_____ _____

Signature Date

Reg Reisenbichler, Research Fishery Biologist

Name and Position Title (print)

Résumé and table for requested take follow.

References

Burton, K., L. Lowe, and H. Berge. 2005. Cedar River Chinook salmon redd and carcass surveys: annual report 2004. Seattle Public Utilities, Seattle, WA.

Stevenson, D. K. and S. E. Campana [ed.]. 1992. Otolith Microstructure Examination and Analysis. Can. Spec. Publ. Fish. Aquat. Sci. no. 117: 130 pp.

Requested annual take by Reisenbichler

ESU/ Species and population group if appropriate	Life Stage	Origin	Take Activity	Number of Fish Requested	Requested Unintentedl Mortality	Research Location	Research Period
PS Chinook Salmon	Juvenile	Naturally Produced	Intentional mortality	70	N/A	Lake Washington	April-July
PS Chinook Salmon	Juvenile	Listed hatchery fish with clipped adipose fin[1]	Intentional mortality	165	N/A	South and Central Puget Sound Basin	June

[1] The hatchery fish will be collected in the hatchery shortly before release and may or may not have clipped adipose fins.

The request for naturally produced fish (first line above) comprises the following details:

Source	Number
North Lake Washington	10
South Lake Washington	30
Bear Cr. (L. Wash. drainage) – trap or seine	10
Cedar River – trap or seine	20
TOTAL	70

Our study requires 35 fish from the Cedar River but we request only 20 because we assume that WDFW will experience at least 15 incidental mortalities of "smolts" at their trap. They have agreed to provide all incidental mortalities to us. If incidental mortalities exceed 15 and are well-distributed through the period of smolt outmigration, we will take accordingly fewer than the 20 fish requested here.

The request for hatchery fish (second line above) comprises the following details:

Hatchery Name	Run	Location	Operated by	# fish
Clear Creek Hatchery*	Fall	Nisqually River	Nisqually Tribe	45
Dungeness/Hurd Creek Hatchery	Spring	Dungeness River	DFW	15
Hupp Springs Hatchery	Spring	White River	DFW	15
Issaquah Hatchery	Fall	Cedar River	DFW	15
Kalama Creek Hatchery	Fall	Nisqually River	Nisqually Tribe	15
Soos Creek Hatchery	Fall	Green River	DFW	15
Voights Creek Hatchery	Fall	Puyallup River	DFW	15
Wallace Hatchery	Summer	Skykomish River	DFW	15
White River Hatchery	Spring	White River	Muckelshoot Tribe	15
			TOTAL # FISH	165

Proposal to the Cedar River Anadromous Fish and Instream Flow Committees
8 March 2006

Title: Analysis of otolith microstructure to assess the importance of lake rearing for Cedar River Chinook salmon (in coordination with microchemistry work by Washington Trout).

Submitted by: Reg Reisenbichler and Kim Larsen; U.S. Geological Survey, Western Fisheries Research Center (WFRC), 6505 NE 65th St, Seattle, WA 98115. Phone: 206-526-6282 x334 (RR) or x232 (KL). email: reg_reisenbichler@usgs.gov; kim_larsen@usgs.gov

CRAFC[1] Research Priority: "(2) What is the relative survival rate to adult for Chinook fry and smolt leaving the Cedar River? – Evaluating a potential method for distinguishing natural origin adults by life history strategy."

Objectives[2]

1. Determine whether otolith microstructure (spacing of daily growth increments on that portion of the otolith reflecting somatic growth in freshwater) differs between Cedar River fish that rear in Lake Washington and those that rear for an extended period in the river before migrating (as "smolts") directly to saltwater. Characterize any difference.
2. Determine whether lake vs. river rearing as juveniles is consistently distinguishable from otoliths of juvenile salmon (2006 and 2007) and adult salmon (2004-2007).
3. Estimate the proportion of Cedar River adults that reared in Lake Washington as juveniles prior to entering Puget Sound. Otoliths from adults will be taken from archived sets (held by WDFW[3]) and from spawning ground surveys during 2006 and 2007. Estimate length of residence and proportion of freshwater growth that occurred in the lake for lake-reared fish.
4. Determine whether circulus spacing on the scales of juvenile Chinook salmon differs between Cedar River fish that rear in Lake Washington and those that rear for an extended period in the river before migrating (as "smolts") directly to saltwater. Characterize any difference.

Background

Juvenile Chinook salmon leave the Cedar River as fry or parr (January-April) and as "smolts" (May-June); however, the subsequent contribution of the former to the adult population is unknown. Knowledge of the relative contributions from lake-rearing fish (the fry or parr migrants) and river-rearing fish to the adult population would be valuable for instream flow management and for prioritization of habitat protection and restoration projects. Otolith analysis provides a tool for developing this information.

Otoliths are calcium carbonate structures in the inner ear that grow in proportion to the overall growth of the fish. Otoliths generally display daily growth increments so that date and fish size at various habitat transitions, and growth rate, can be estimated by back-calculation. For example, similar work in the Skagit River shows that otolith microstructure yields the number of days that a fish resided in the estuary as a juvenile, size at entrance to the estuary, and the amount that the fish grew while in the estuary. We expect similar success here because of substantial growth differences between lake and river (Dave Beauchamp, personal communication).

Assessing life history strategies through otolith analysis is valuable because it can obviate the need for traditional mark-recapture methods that are extremely expensive, often biased or infeasible, and do not directly reveal the importance for adult recruitment (i.e., they do not account for differential survival after fish enter Puget Sound or the ocean). The contribution to adult production defines the value of different life history strategies to the population, and provides a key component for estimating stage-specific survivals if life-cycle modeling is desired. Analysis of otolith microstructure for these purposes, focused on the use and importance of

[1] CRAFC = Cedar River Anadromous Fish Committee; IFC = Instream Flow Committee
[2] Washington Trout, in a sister proposal dated 16 February 2006, proposes a complementary set of objectives, based on analysis of otolith microchemistry.
[3] Washington Department of Fish and Wildlife

estuary habitats, is proving highly successful in a similar study that USGS and Skagit River System Cooperative are conducting at the Skagit River in northern Puget Sound (work is in progress and the corresponding report is in preparation). Neilsen et al. (1985) analyzed otoliths to evaluate estuary use by juvenile Chinook salmon in Oregon's Sixes River, and we are doing the same for Skagit River Chinook salmon.

Scales are similar to otoliths in that they provide a record of fish growth throughout most of a fish's life; however in contrast to otoliths, the deposition rate for increments (circulli) is not constant but varies with growth rate and therefore provides reduced sensitivity for detecting growth differences. Resorption of the scales on mature fish also can be problematic. Nevertheless, the cost of analysis is much less for scales than for otoliths, making scale analysis an attractive option if it is effective. We will test whether and to what extent cirullus spacing differs between the lake-reared and the river-reared fish collected for otolith analysis. Reimers (1973) analyzed scales to evaluate estuary use by juvenile Chinook salmon in Oregon's Sixes River.

Scope of work

This proposal accommodates a sister project (separate proposal) by Washington Trout who propose microchemical analysis of the same set of otoliths to be acquired and used in this study. Together these projects evaluate microstructure and microchemistry simultaneously, thereby providing efficiencies in otolith preparation and providing a prompt answer for whether either method can provide the desired information on Chinook salmon life history. The microstructure analyses (USGS) require more careful preparation of otoliths than does microchemistry and the same otoliths will be evaluated by both methods, therefore Washington Trout and we have agreed that USGS will prepare the otoliths for both analyses.

During the first year (2006) we will acquire otoliths from juvenile and adult Chinook salmon from the Cedar River/Lake Washington system. We will prepare and examine otoliths from the juveniles collected this year to assess the likelihood of successfully achieving Objective 3 through microstructure analysis. Similarly, Washington Trout during 2006 will use the same otoliths to assess the likelihood of success with the microchemistry analysis (see Washington Trout's proposal). If otolith microstructure appears unlikely to provide the necessary discriminatory power, that work will be terminated and we will prepare and submit a final report of our results early in 2007. If microchemistry analysis were to continue, we would transfer all samples to Washington Trout.

Objective 1 involves processing the otoliths from more than 105 wild juveniles during October 2006 through March 2007. The actual number will depend on how much incidental mortality occurs at the Bear Creek trap. Juveniles will be collected according to the following schedule.

Source	Jan-Apr	Apr	May/Jun
North Lake	--	10	--
South Lake	--	30	--
Cedar River trap	30	--	35
Bear Cr. Trap	(morts)	(morts)	(morts)
Hatcheries	--	30	--
Total wild			105
Total			135

The juvenile salmon will be collected from Lake Washington by beach seining, and perhaps snorkeling, by or with assistance from Roger Tabor (U.S. Fish and Wildlife Service), and from the traps in Cedar and Bear Creeks courtesy of Greg Volkhardt (Washington Department of Fish and Wildlife [WDFW]). As much as possible, these fish will be accidental mortalities from

trapping or other operations. Juvenile fish also will be requested from the two hatcheries to determine the likelihood of identifying and excluding unmarked hatchery fish from among adults taken from Cedar River spawning areas. Each fish will be measured for length and weight, euthanized (if not already dead), and preserved in alcohol, on ice, or frozen. The preserved fish will be taken to WFRC where the otoliths will be extracted, mounted, ground, and polished according to our standard protocols for viewing daily increments. The increments and freshwater growth zones in the otolith microstructure will be measured by microscope and digital image analysis system. Standard statistical techniques will be used to compare the increment spacing for lake-caught and river caught fish, with particular attention to the extent, if any, of overlap in these characters.

If the growth patterns for lake- and river-reared fish are found to be distinct with minimal if any overlap, we propose to collect and process additional juvenile fish in 2007 to assess interannual variability (Objective 2), and we will process the otoliths from at least 135 adult Chinook salmon (carcasses; Objective 3). Adult fish will be sampled by carcass survey crews in the Cedar River during fall of 2006 and 2007. Additional otoliths from adults will be analyzed from collections archived by WDFW in previous years (see above), recognizing that some error might be introduced due to interannual variation in relative growth between river and lake. We expect this error to be negligible[4]. The number of otoliths analyzed will be approximately equal from each of the available return years. We will initiate coordination with carcass sampling crews no later than June 2006 and will request otoliths from at least 100 adult salmon, any excess above our needs will be archived. As noted above, otoliths from adults are extremely valuable because they reveal the life histories of successful fish – fish that survived to reproduce.

Work will begin as soon as we are notified that the study has been approved for funding. We anticipate that Greg Volkhardt (WDFW) will begin preserving incidental mortalities at the traps even before we are notified. We will present an oral report of progress to CRAFC and IFC or their representatives in February 2007 to inform them whether, from the initial subsample, growth and otolith microstructure in Lake Washington differ sufficiently from those in the Cedar River to provide suitable discrimination. Data analysis and report preparation for juvenile fish will continue through April 2007, and a report describing the results from the juveniles collected during 2006 will be sent out for peer review no later than May 2007. A written report, reflecting the peer review, should be delivered to CRAFC by the end of July 2007. During the second year (2007) we will follow the 2006 schedule for juvenile collections, complete analysis of the otoliths from these fish and any remaining from 2006, and complete work on the otoliths from adults. Analysis of scales from juvenile fish will occur during 2007 or early 2008. Sample and data analysis and report preparation will continue into 2008. We anticipate delivery of the final peer-reviewed report (for both years) in March 2008.

Deliverables

Product	Delivery Date
Oral report on results for juveniles collected during 2006	February 2007
Written report on results for juveniles collected during 2006	July 2007
Final report	March 2008

Duration: April 1, 2006 to March 31, 2008.

[4] The most rigorous analysis would involve comparison of adults and juveniles from the same (e.g., 2005) year-class so that interannual variation could not be a confounding factor.

Potential management implications of results: Knowledge of the relative contributions from lake-rearing fish (the fry migrants) and river-rearing fish to the adult population will be valuable for instream flow management and for assigning priorities to habitat protection and restoration projects.

References

Bacon, C.R., P.K. Weber, K.A. Larsen, R.R. Reisenbichler, J.A. Fitzpatrick, and J.L. Wooden. 2004. Migration and rearing histories of chinook salmon (Oncorhynchus tshawytscha) determined by ion microprobe Sr isotope and Sr/Ca transects of otoliths. Canadian Journal of Fisheries and Aquatic Sciences 61(12): 2425-2439.

Neilson, J.D., G.H. Geen, and D. Bottom. 1985. Estuarine growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*) as inferred from otolith microstructure. Canadian Journal of Fisheries and Aquatic Sciences 42:899-908.

Reimers, P.E. 1973. The length of residence of juvenile fall Chinook salmon in Sixes River, Oregon. Research Reports of the Fish Commission of Oregon 4(2):1-43

TITLE: Pre-restoration habitat use by Chinook salmon in the Nisqually estuary using otolith analysis, Nisqually NWR.

2. FWS PROJECT OFFICER: Marian Bailey, Refuge Biologist, Nisqually NWR, 100 Brown Farm Road, Olympia, WA 98516. V: 360-753-9467, F: 360-534-9302, marian_bailey@fws.gov

3. USGS CONTACT OR SUGGESTED PRINCIPAL INVESTIGATOR: Reg Reisenbichler, Fishery Biologist, Western Fisheries Research Center (WFRC), U.S. Geological Survey, 6505 NE 65th St,. Seattle, WA 98115. V: 206-526-6559, F: 206-526-6654, reg_reisenbichler@usgs.gov.

4. FWS REGIONAL RESEARCH COORDINATOR: Paul Heimowitz, Region 1 Research Coordinator, FWS Region 1 Office-6E 911 NE 11th Ave Portland, OR 97232. V: 503-736-4722, paul_heimowitz@fws.gov

5. PARTNERSHIPS AND ROLES: Jean Takekawa, Refuge Manager, Nisqually NWR, 100 Brown Farm Road, Olympia, WA 98516. V: 360-753-9467, F: 360-534-9302, jean_Takekawa@fws.gov. The Refuge contributes expertise and in-kind support by directing and coordinating the field portion of the study, providing supervision, operative support equipment and supplies.

David Trout, Natural Resources Director, Nisqually Indian Tribe, 12501 Yelm Highway SE Olympia, WA 98513. V: 360-438-8687, F: 360-438-8742**,** dtrout@nwifc.org The Tribe is providing in-kind contributions including a skilled fish biologist, fish technicians, and equipment including a boat, a seine, and fyke nets to the otolith study.

Dan Golner, Regional Biologist, Ducks Unlimited Pacific Northwest Field Office, 1101 SE Tech Center Drive, Suite 115 Vancouver, WA 98683. V: 360-885-2011 x 17, F: 360-885-2088, dgolner@ducks.org DU provides in-kind contributions and works through an IDIQ contract.

Reg Reisenbichler, Fishery Biologist. See #3. USGS PRINCIPAL INVESTIGATOR. USGS will assist with project planning and oversight, provide training and oversee technical aspects of the otolith microstructure analysis, and provide data analysis and interpretation of the results. USGS will receive the proposal funding.

Ken Burg, Project Leader, Western Washington Fish and Wildlife Office Ecological Services Office (Ecological Services), 510 Desmond Dr. SE Suite 102 Lacey, WA 98503 V: 360-753-6039, F: 360-753-9405, ken_berg@fws.gov. Ecological Services has provided expertise, in-kind contributions and fund transfers.

Bob Wunderlich, Supervisory Fishery Biologist, Ecological Services-Fisheries, 510 Desmond Dr. SE Suite 102 Lacey, WA 98503. V: 360-753-6039, F: 360-753-9008, bob_wunderlich@fws.gov. The Ecological Services Fisheries program has provided expertise and loaned equipment.

Kyle Brakensiek, Salmon Ecologist with Northwest Indian Fisheries Commission, 6730 Martin Way E., Olympia, WA 98516. V: 360-528-4302, F: 360-753-8659, kbrakensiek@nwifc.org. Included the Refuge under the Nisqually Tribes NOAA take exemption.

This study will provide data needed to evaluate the estuary restoration planned by the Refuge and is based on a very strong partnership between Nisqually NWR, the Nisqually Indian Tribe, Ducks Unlimited, USGS, and the Northwest Indian Fish Commission. It also has USFWS cross program support and technical assistance from Refuges, Fisheries, and Ecological Services.

The recently approved Nisqually NWR Comprehensive Conservation Plan (CCP) identified a 699-acre estuary restoration project as a top priority. Pre and post restoration monitoring is listed as a high priority in the CCP and is critical to interpret the response of fish and wildlife to restoration. We believe that a rigorous study of Chinook otoliths is a crucial component of this monitoring priority. The proposed otolith study will also help to meet monitoring priorities listed in the 2001 Nisqually Chinook Recovery Plan, namely monitoring the development of a self-sustaining stock and its developing life history diversity.

The strong partnership with the Nisqually Tribe has resulted in an ongoing, comprehensive fish study that includes otolith analysis. Ducks Unlimited fully supports our partnership and has hired a full time fish biologist and a seasonal fish technician (working out of the Refuge office) to conduct the comprehensive fish characterization study on the Nisqually estuary. Chinook salmon collections make otoliths available to USGS for analysis. USGS also benefits because the study satisfies various elements of the USGS mission by providing assistance to (1) a sister agency (USFWS) and to (2) a Native American Tribe for (3) an extremely valuable ecosystem restoration project that (4) should benefit an ESA-listed species (Chinook salmon). The Western Washington Fish and Wildlife Office of Ecological Services (Ecological Services) has provided technical support and funding because it will provide needed information regarding federally listed Chinook life histories to estuary habitat use and supports efforts to determine how restoration efforts will enhance this fish species. The Ecological Services Fisheries program has shown support for this project by providing original study design, a coded-wire tag reader each field season and briefly they loaned a field boat to the Refuge. This project will shed light on habitat use of hatchery Puget Sound Chinook relative to wild Puget Sound Chinook and will give fishery managers greater understanding how to support the wild Chinook of Nisqually River. Both the Northwest Indian Fisheries Commission and the Nisqually Tribe support this project by including the Refuge on the Tribes NOAA take exemption to collect a number of juvenile Chinook for the otolith study.

6. TYPE OF SUPPORT REQUESTED: Technical Assistance

7. PROBLEM STATEMENT AND IMPLICATIONS: The Nisqually Fall Chinook stock is one of the 27 stocks in the Puget Sound evolutionarily significant unit listed as threatened under the Endangered Species Act. The preservation of the Nisqually delta ecosystem coupled with extensive restoration of approximately 1000 acres of diked estuarine habitat is identified as the highest priority habitat action for the recovery of naturally spawning, self-sustaining Nisqually River fall Chinook (*Oncorhynchus tshawytscha*) in the Nisqually Chinook Recovery Plan.

In order to evaluate the response of Chinook salmon to restoration, a pre-restoration baseline of life history diversity and estuary utilization must be established. Otolith analysis has been identified as the best way to measure Chinook salmon life history diversity, growth, and residency in the Nisqually estuary. Over time, the information from the otolith analyses will be used to: 1) determine if estuary restoration changes the composition of life history trajectories of Nisqually River Chinook, 2) compare pre and post restoration residence times and growth rates, and 3) validate local and regional habitat restoration priorities.

8. OBJECTIVES: The purpose of this study is to evaluate and use otolith microstructure analysis as a tool for characterizing the importance of the estuary to Chinook salmon in the Nisqually River before and after restoration at Nisqually NWR. This tool would be used to quantify use of this habitat, thereby providing a partial assessment of restoration benefits to federally threatened Chinook salmon.

Otoliths are calcium carbonate structures in the inner ear that grow in proportion to the overall growth of the fish. Otoliths generally display daily growth increments so that date and fish size at various habitat transitions can be back-calculated. Careful analysis of otolith microstructure yields the number of days that a fish resided in the estuary as a juvenile, size at entrance to the estuary, size at egress, and the amount that the fish grew while in the estuary.

Juvenile Chinook salmon exhibit three basic life history trajectories – some enter the sea (or Puget Sound) as fry, some rear in the estuary before entering the sea, and some rear in the river until reaching lengths of ~80 mm or greater and then move rapidly through the estuary into the sea as smolts. Presumably successful restoration will increase the number of salmon that rear in the estuary, or residence and growth for individual salmon in the estuary. A large number of hatchery Chinook salmon occur in the Nisqually River, so it is important to evaluate estuary utilization separately for hatchery and wild fish.

We employ otolith microstructure because traditional mark-recapture methods are extremely expensive or inadequate in estuary habitats, typically are biased and substantially underestimate use, and do not directly reveal the importance for adult recruitment (i.e., they do not account for differential survival afterward in Puget Sound or the ocean). Analysis of otolith microstructure for these purposes, while new, is proving highly successful in a similar study that USGS and partners are conducting in the Skagit River system located further north in Puget Sound which can serve as a reference site for the before/after comparison in the Nisqually River. The objectives are:

1. Develop a Nisqually specific signature of otolith microstructure growth patterns and checks that allow us to distinguish growth and residence of juvenile salmon in the estuary from growth in the river

(upstream) and in Puget Sound (seaward). Evaluate between-year variation in these characters by comparing otoliths collected in 2004 with those collected in 2005.

2. Determine whether distinct growth patterns on the otoliths of hatchery and wild salmon in the Nisqually River allow us to recognize unmarked hatchery fish and separate them from wild fish.
3. Analyze the otoliths of returning adults in order to catalog the juvenile life-history trajectories of the "successful" fish and provide a preliminary estimate for the proportions and numbers of wild and hatchery adults that reared in the delta and estuary as juveniles.
4. Describe the relationship between juvenile Chinook size and/or date of entry to the estuary with Chinook growth rate and/or residence time in the estuary.

9. METHODS AND STUDY AREA: Up to 250 wild and 250 hatchery juvenile Chinook will be collected from February – October in 2005 to add to the approximately 200 wild and 200 hatchery Chinook collected in 2004. The Chinook are collected by beach seining and fyke trapping in the following distinct habitat zones: 1) freshwater, 2) forested riverine tidal, 3) emergent forested transition, 4) estuarine emergent marsh, 5) mud flat, and 6) nearshore. Each fish will be euthanized, measured for length and weight, and its otolith extracted. The otoliths will be taken to WFRC where they will be mounted, sectioned, and polished according to standard protocols. The various increments, checks, and growth zones in the otolith microstructure will be measured by microscope and digital image analysis system. Our work from the Skagit River indicates that recognizable checks will indicate the time (and size) that a fish enters the estuary and delta and the time of entrance to Puget Sound. We will test whether Nisqually fish consistently display such checks, and identify the geographic locations associated with the checks. Microchemistry assays for Strontium/Calcium on the otolith may be included as an additional validation of entry to the brackish portion of the estuary.

Carcass survey crews, primarily from the Nisqually Tribe and Washington Department of Fish and Wildlife, will remove the head or otoliths from the carcasses of adult salmon returning to the Nisqually River. These otoliths are extremely valuable because they reveal the successful life histories – those that succeeded in producing mature adults. The actual number of adults examined will depend on the time/funds available after processing the otoliths from juvenile fish. The remaining otoliths from adults will be archived and held for future analysis and comparison with adults collected after restoration is completed.

10. PROJECT DURATION: The project will start on 1 January 2006 and end 15 December 2006.

11. PRIORITY: The Nisqually Refuge is currently implementing the largest estuarine restoration project in the Pacific Northwest. This study supports pre and post restoration monitoring that is essential to evaluate the success of restoration and allows for adaptive management. This information will also be invaluable as a resource for other estuarine restoration projects in the Pacific Northwest.

We believe this rigorous study of Chinook otoliths is a crucial component to establishing baseline use of the existing conditions. The proposed otolith study will help meet not only USFWS monitoring priorities but also monitoring priorities listed in the 2001 Nisqually Chinook Recovery Plan, namely monitoring the development of a self-sustaining stock and its developing life history diversity.

Habitat restoration priorities in the 2005 Shared Strategy Draft Puget Sound Salmon Recovery Plan are based on the assumed presence of multiple Chinook life history types throughout Puget Sound. This study will help verify these priorities by documenting current diversity and will provide a template for monitoring recovery over time.

This otolith project will assist federal, state, and tribe salmon fishery managers throughout the Pacific Northwest to better compare and understand life histories of hatchery and wild Chinook salmon. Additionally, study results, along with further analyses, will be highly valuable for the Tribe's Chinook recovery planning.

12. PRODUCTS AND SCHEDULE: Products will be a) a written interim report due July 15, b) a written, final report of the results and c) a copy of the raw data that will be delivered to the cooperators (U.S. Fish and Wildlife Service and Nisqually Tribe) by 15 December 2006.

13. BUDGET: See separate page.

14. APPROVALS AND SUBMITTAL: Not yet required.